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Ecosystem partitioning of ^{15}N-glycine after long-term climate and nutrient manipulations, plant clipping and addition of labile carbon in a subarctic heath tundra

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ABSTRACT

Low temperatures and high soil moisture restrict cycling of organic matter in arctic soils, but also substrate quality, i.e. labile carbon (C) availability, exerts control on microbial activity. Plant exudation of labile C may facilitate microbial growth and enhance microbial immobilization of nitrogen (N). Here, we studied ^{15}N label incorporation into microbes, plants and soil N pools after both long-term (12 years) climate manipulation and nutrient addition, plant clipping and a pulse-addition of labile C to the soil, in order to gain information on interactions among soil N and C pools, microorganisms and plants. There were few effects of long-term warming and fertilization on soil and plant pools. However, fertilization increased soil and plant N pools and increased pool dilution of the added ^{15}N label. In all treatments, microbes immobilized a major part of the added ^{15}N shortly after label addition. However, plants exerted control on the soil inorganic N concentrations and recovery of total dissolved ^{15}N (TD^{15}N), and likewise the microbes reduced these soil pools, but only when fed with labile C. Soil microbes in clipped plots were primarily C limited, and the findings of reduced N availability, both in the presence of plants and with the combined treatment of plant clipping and addition of sugar, suggest that the plant control of soil N pools was not solely due to plant uptake of soil N, but also partially caused by plants feeding labile C to the soil microbes, which enhanced their immobilization power. Hence, the cycling of N in subarctic heath tundra is strongly influenced by alternating release and immobilization by microorganisms, which on the other hand seems to be less affected by long-term warming than by addition or removal of sources of labile C.

1. Introduction

The Arctic, covering 5% of the terrestrial surface, contains 14% of the global carbon (C) stocks, mainly bound in soil organic matter (SOM) (Post et al., 1982). Moreover, the SOM contains large pools of nutrients, e.g. nitrogen and phosphorus (P). In subarctic, montane heath ecosystems, the SOM pool contained 78, 83 and 59% of the C, N and P ecosystem pools, respectively, and the microbial pool made up 2.5, 6.5 and 30% of the C, N and P pools, respectively (Jonasson et al., 1999a). Hence, the cycling of carbon and nutrients is tightly linked, and microbial decomposition of SOM is the bottleneck for inorganic nutrient availability in Arctic soils (Van Cleve and Alexander, 1981; Post et al., 1982). The microbial decomposition of organic matter is restricted by low temperatures and high soil moisture (Flanagan and Veum, 1974; Heal et al., 1981), and predicted climate warming of 3–5 °C in the Arctic within the next century (IPPC, 2001; ACIA, 2005) could accelerate SOM decomposition and release of inorganic nutrients to the soil matrix. However, the recalcitrant nature of SOM, with high C:N ratio and most C bound in complex organic compounds as lignin, which is difficult to degrade, can lead to C limitation of microbial activity, because most C present in the soil pool is not easily available. Addition of labile C sources to tundra soils leads to increased microbial biomass C and respiratory activity. Furthermore, labile C increased microbial nutrient demand (Jonasson et al., 1996b; Illeris and Jonasson, 1999), which has a negative influence on plant growth due to lower plant nutrient availability (Schmidt et al., 1997a; Schmidt et al., 1997b). Thereby not only climatic factors such as temperature and soil moisture, but also the properties of the substrate restrict the microbial SOM cycling.

Vegetation exerts influence on the microbial community both because plants provide organic material to the soil and because they at the same time compete with the microbes for nutrients. Plant roots exude compounds rich in labile C to the soil matrix by leaching and root death, and thereby exert control on microbial activity, because the microbial community is often limited by C

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substrate availability (Wardle, 1992; Priha et al., 1999; Jones, 2004). Plants also influence rates of microbial decomposition rates through the quantity and quality of litter produced (Hobbie, 1992; Hobbie, 1995; Chapman et al., 2006). Microbes compete with plants for nutrients (Schimel and Bennet, 2004), and because plants have longer turnover rates than microbes, they can compete for the same N several times (Kaye and Hart, 1997). Plant species differ in their ability to compete with microbes by taking up nutrients from the soil at various rates (Priha and Smolander, 2003; Templer et al., 2003), and with species specific N-form preferences (Nordin et al., 2001). Also, research in the last decade has shown that plants can take up organic N directly and thereby short circuit the microbial mineralization cycle (e.g. Chapin et al., 1993; Näsholm et al., 1998). In the Arctic the widespread presence of mycorrhizal symbiosis complicates the relationship between plants and microbes further. Plant roots support mycorrhizal growth by transfer of labile C to the mycorrhizal fungi, and in return the plants receive nutrients (Smith and Read, 1997).

In order to gain knowledge on interactions among soil nutrients, microbes and plants, we manipulated the ecosystem by long-term warming and fertilization, and also altered soil substrate availability by removing plants and by adding a labile C source to the soil. We performed the experiment at a site where manipulations of temperature and nutrient availability have been done since 1989 in order to investigate effects of future climatic warming and to obtain a broader knowledge on ecosystem processes when relieved from N-limitation (Jonasson et al., 1999b). For 3 years prior to this study, a part of each plot was kept free of vegetation by clipping, and in a part of the clipped plots, we subsequently added a labile C source (sugar). Glycine (^{15}N-labelled) was injected into the plots, and changes in the relationship between the uptake of N between the microbial biomass and the plants as a consequence of higher temperature or fertilizer addition were analysed. The degree of interaction between N uptake by plants and microbes was quantified based on an analysis of the ^{15}N uptake in microbes in clipped and unclipped plots. We hypothesized that plant clipping would reduce the amount of soil labile C, and hence, microbial C limitation would increase. The aim of the addition of sugar was to reveal whether the microbial uptake of N is dependent on the amount of easily degradable carbon in the soil. We hypothesized that microbial N uptake would increase in plots with added labile C due to enhanced potential for microbial nutrient immobilization. The long-term pre-treatments of warming and fertilizer addition were expected to increase nutrient availability in the soil, and thereby reduce the severity of plant–microbial competition for nutrients and added ^{15}N.

2. Materials and methods

2.1. Site description and experimental design

The experiment took place at a site near Abisko in northern Swedish Lapland, in a sub alpine heath just above the forest line at 450 m above sea level. The climate at the site is subarctic, with a growing season of approximately 3 months lasting from mid-June to early–mid-September; see Havström et al. (1993) and Michelsen et al. (1996a) for more detailed accounts on climate and vegetation.

The experimental setup of the site was initiated in 1989. It consisted of four long-term pre-treatments: control, warming, fertilizer and warming + fertilizer. The pre-treatments were replicated across six blocks within an area of ~800 m^2. Each year in early June, just after snowmelt, and until the end of August or early September, the temperature was raised by erecting dome-shaped open top plastic greenhouses with a 1.2 × 1.2 m surface area. The greenhouses enhanced the air and soil temperature by 3.9 °C and 1.2–1.8 °C, respectively (Havström et al., 1993; Michelsen et al., 1996a). For further details on pre-treatment effects on air, soil and plant shoot temperatures; see Havström et al. (1993) and Jonasson et al. (1996b). Fertilizer addition simulating enhanced nutrient availability due to accelerated mineralization after warming was done each year soon after snowmelt in June at a rate of 4.9 g m^{-2} for N and 1.3 g m^{-2} for P in 1989. From 1990 to 2000, the additions were 10.0 and 2.6 g m^{-2}, respectively. No fertilizer was applied in 1993, 1998 and 2001, but fertilizer was added on 16 June 2002, 1 month prior to our last sampling.

In order to manipulate the availability of C substrate in the soil, we removed the vegetation in July 1998 by clipping a subplot within each pre-treatment plot and any resprouting vegetation was clipped off during the 1999 and 2000 growing seasons. In July 2001, three subplots each of 20 by 20 cm were assigned within each pre-treated plot. The subplots were delimited by steel-frames that were pressed c. 10 cm into the ground. One plot was with aboveground vegetation retained (unclipped − sugar). The second plot was with vegetation clipped since 1998 (clipped − sugar), and the third plot was with vegetation clipped since 1998 plus addition of 10 g sugar corresponding to 250 g m^{-2} (clipped + sugar). The sugar, added on 23 July 2001, was dissolved in 250 ml water, and 250 ml water also was added to the non-sugar amended plots. The clipping and sugar addition, with the purpose of changing substrate quantity and quality, is designated substrate treatment in the text.

The ^{15}N label addition took place on 24 July 2001, i.e. 1 day after the sugar addition, as a pulse labelling to the soil with ^{15}N-glycine. The label was injected with a syringe guided by a grid frame with 25 holes and with each point receiving 10 ml; in total 250 ml for each plot. Each plot was labelled with ^{15}N-glycine (98%, Cambridge Isotope Laboratory) equal to 0.128 g N m^{-2}, and the label was evenly distributed to the soil column from just below the green moss mat to a depth of ~5 cm.

2.2. Sampling and analyses of plants and soil

On 25 July, 27 July and 24 August 2001, and on 21 July 2002, i.e. 1 day, 3 days, 1 month and 1 year after label addition, two soil cores of 4 cm diameter were collected from the organic horizon to a depth of 6 cm in each plot and pooled. On 21 July 2002, we further sampled two soil cores in each plot to a depth of 12 cm in order to detect any movement of label to deeper soil horizons. Roots were sorted out of the soil with tweezers, and washed several times with 0.5 mM $CaCl_2$ to remove any adhering label. Ten grams sorted, fresh soil was fumigated with $CHCl_3$ for 24 h to release the N and C in the soil microbial biomass, after which the soil was extracted for 1 h in 50 ml 0.4 M K_2SO_4 (Brooks et al., 1985). The extracts were filtered through Whatman GF-D filters and frozen until analysis. Another 10 g fresh soil was treated as above, but without the $CHCl_3$ fumigation to recover soil inorganic N. Part of the extracts was analysed for NH_4^+-N by the indophenol method and for NO_3^--N by the cadmium reduction method (Allen, 1989). To obtain microbial N and C, 2.5 ml of fumigated and unfumigated K_2SO_4 extract was digested with 1.25 ml concentrated H_2SO_4 with H_2SeO_3 and 0.25 ml 30% H_2O_2 added, after which distilled water was added to a volume of 25 ml. The N content was analysed by the indophenol method and the C content with a Shimadzu Total Organic Carbon Analyzer. DON was determined as the N content in digested, unfumigated extracts after subtraction of the inorganic N fraction, and TDN was defined as the total N content of digested unfumigated extracts, i.e. soluble organic N plus inorganic N. DOC is reported as the content of organic carbon in unfumigated extracts. Microbial N and C were calculated by subtracting the N or C, respectively, in digested, unfumigated extracts from the content in digested, fumigated extracts. The microbial N was calculated using an extractability factor (K_{EN}) of 0.4 and the microbial C by a K_{EC} factor of 0.45 (Schmidt et al., 2002).